Fall '12 **CH370** Name _____
Hackert **HW- 2 (20 pts)** UTeID _____

1. The absorbance of UV light at 280 nm by proteins is mostly due to the aromatic amino acids tyrosine and tryptophan. Lactate DH monomers (36,507 Da) have 332 a.a. and contain 5 residues of tryptophan and 7 residues of tyrosine. Tetrameric LDH has a molar extinction coefficient of 137,450 $M^{-1}cm^{-1}$ at 280 nm. A sample in a standard 0.50 cm cuvette was found to have a T of 59% at a wavelength of 280 nm.

   (1) a) What is the **absorbance** for this sample protein solution? _____

   (1) b) Calculate the **E(1%)** extinction coefficient for this protein at 280 nm. _____

   (1) c) Calculate the concentration of this protein solution in **mg/mL** _____

2. Consider a FRET experiment where the measured efficiency of energy transfer between two chromophores is 45.5%. If R_o = 32.0 Å, **estimate the separation** of the two chromophores. **(R = _____)**
   (1)

3. Balance the following radioactive decay equation by filling in the blank with the missing item.

   (1) a) $^{206}Tl \rightarrow$ ____ + β^-

   (1) b) A radioisotope has a **rate constant** of **0.027 / yr**. Calculate the **half-life** of the radioisotope**.**
   Half-life = _____

   (1) c)How many **years** will it take for 90% of a sample of this radioisotope rated at 35 microCuries to undergo radioactive decay ? _____ **years.**

4. SDS gels are greatly improved in resolution by running a "stacking" gel and a "resolving" or "running" gel.
   a) **Name two key property differences** between the "stacking" gel and the "resolving" gel that contribute to the improved resolution of running DISC PAGE.
   (1) a)

   b)

   What is the role of each of the following in performing SDS-PAGE?
   a) Bromophenol Blue
   (1)
   b) Coomassie Blue:

5. The equation of motion for a small, spherical particle of mass (m) and frictional coefficient (f) that is initially at rest, and then acted on by a constant force (F) at time t = 0 is F –fv = ma. (From calculus recall that F - fv = m(dv/dt) solves to v = (F/f) [1 - exp(-ft/m)].)
   a) Show that such a particle will initially accelerate but over time will approach a "maximal" velocity.
   (1)

   (1) b) Consider protein molecule that is assumed to be spherical with a diameter of 75Å, a density of 1.35 g/cm^3 and a v-bar of 0.73 cm^3/g. Calculate the expected diffusion constant for this protein (Assume T = 20^o C and η = 0.01 (g/cm-s).

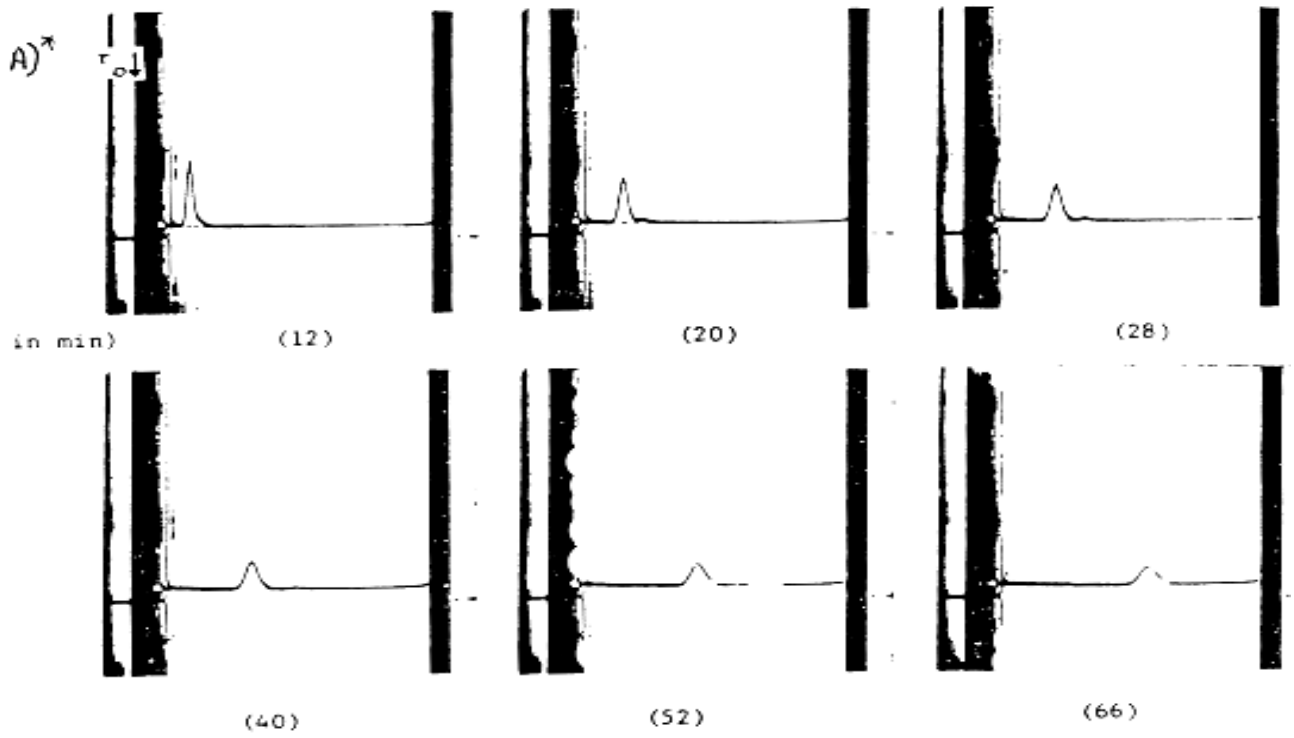
6. What is typically measured by dynamic light scattering (LS)? _____
   (1)
   What wavelengths are normally employed in making circular dichroism (CD) spectra? _____

7. Determine the sedimentation coefficient (s) and molecular weight (M) for the sample that gave the following data when subjected to: A) a sedimentation velocity run using Schlieren optics, and B) a sedimentation equilibrium run using interference optics.

Note: the figures below have been magnified to allow you to make measurements from the figures. The "r" can be determined from the reference points (r_o) and the magnification factors. Assume T = 20° C, density of buffer = 0.9978 g/mL, and v-bar = 0.737 cm^3/g for the protein, and η = 0.01 (g/cm-s) for both experiments.

A) Sed. Vel. : ω = 40,000 rpm, magnification factor (2.5X), r_o = 5.72 cm. (times are given in minutes).

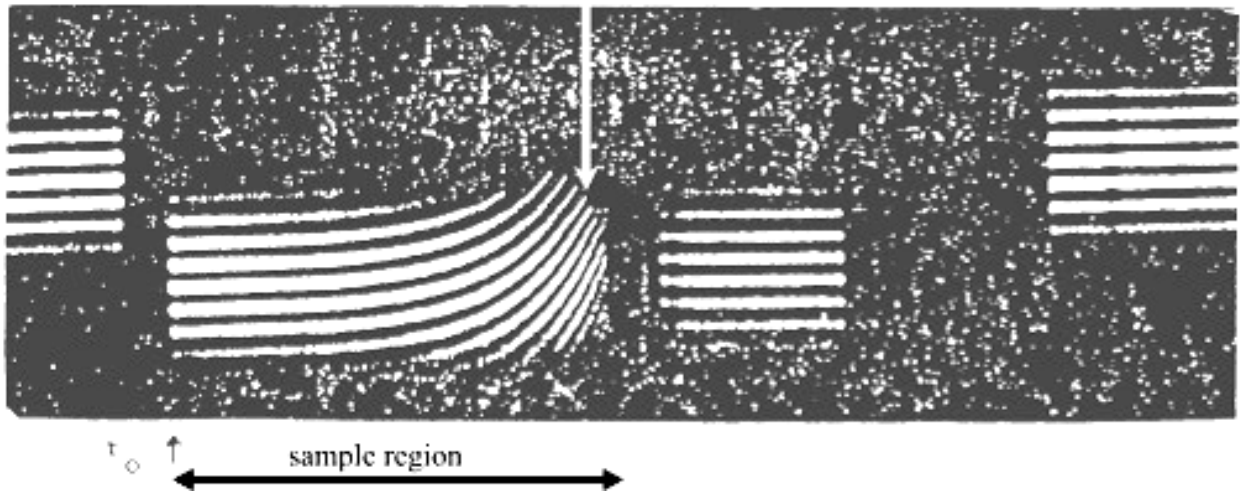
(4) Report "s" in proper units [s = _____] (Show work and attach plot).



B) Sed Equilibrium: ω = 5200 rpm, magnification factor (25X), r_o = 6.75 cm. Calculate M in g/mol (4pts) and

(4) also estimate the concentration of the protein at the position with the white arrow (1 pt). Assume the cell path length to be 12.00 mm, λ = 546 nm, and (dn/dc = 0.186 $(g/cm^3)^{-1}$.

[M = _____ ; [ ] $_{arrow}$ = _____] (Show work and attach plot).



I hereby declare that I did this assignment independently: _____